HYBRID PRODUCTION SYSTEMS BASED ON SELF-INCOMPATIBILITY IN OILSEED *BRASSICA*

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INTRODUCTION

Self-incompatibility (si) in *Brassica napus* is controlled by at least two multi-allelic loci inherited from the progenitor species, *B. oleracea* and *B. rapa*. While self-incompatibility in these ancestral species is a naturally occurring system to enforce outcrossing, in the allotetraploid, self-compatible (sc) *B. napus* the s-loci while functioning do not produce an si phenotype. Self-incompatibility already plays a major role in commercial hybrid production of vegetable Brassicas *(B. oleracea)* and Chinese cabbage *(B. rapa)*. When si is introduced into *B. napus* expression can vary with alleles, genotype and environment. The utilization of si for an efficient and predictable hybrid production system in oilseed *B. napus* depends heavily on the selection of self-incompatibility alleles (s-alleles) that consistently 1) exhibit strong si expression for pollination control, 2) deliver a high level of hybridity in various genotypes and environments, and 3) provide easy s-line maintainability.

Although there is no question that commercially valuable hybrids are potentially attainable, early suggestions that hybrid canola can achieve 40-60% seed yield advantage over cultivars has yet to be realized (Sernyk and Stephansson, 1983). Hybrids based on self-incompatibility produced by pollen manipulation indoors, have showed very promising results in small scale experimental field plots simulating hybrid yield trials. Yield performance at least as good as the best cultivars (Esch and Wricke, 1995) or 20% higher than the higher yielding parent have been reported (Banks and Beversdorf, 1994). Similarly, currently available data on hybridity level in *B. napus* in experimental field trials utilizing si indicate that levels over 90% are readily attainable. To estimate selfing, leaf traits, erucic acid levels (Fu *et al.*, 1995) and petal traits (Werner *et al.*, 1995) were used as markers.

ORIGIN OF SELF-INCOMPATIBILITY IN *B. NAPUS*

B. oleracea and *B. rapa* contain a minimum of 50 and 35 alleles at the s-locus, respectively (Okendon, 1974). Functional s-alleles can be introduced into *B. napus* background by resynthesis, selection or introgression. Resynthesis involves the *de novo* hybridization of the ancestral species. Ovule culture of interspecific hybrids of *B. rapa* x *B. oleracea* (Thompson *et al.*, 1983; Plumper, 1991), and testcrosses to parents have shown that s-alleles from both ancestors are active in such hybrids (Beschorner and Odenbach, 1991).

Information on s-alleles selected and identified from *B. napus* winter oilseed populations indicate that the natural occurrence of si incidence is very low and ranges from 0.08-6.6% (Esch and Wricke, 1995; Fu *et al.*, 1995; Kučera *et al.*, 1995). Self-incompatibility was identified in individuals with low fertility during screening of large populations. In general these s-alleles behave like recessive. S-alleles loosely fall into three groups, namely strong, intermediate and weak alleles. Strong alleles are usually dominant over weak alleles, while many others are intermediate in expression and have varied interactions among themselves. Due to the inherent weakness in expression,

plants with recessive alleles tend to be more easily maintainable by selfing with CO_2 exposure or salt solution, than those carrying stronger, dominant alleles. Since in the classical sense, these s-alleles cannot be considered neither recessive nor dominant, a current view is to refer to them as either 'suppressible' (recessive) or 'non-suppressible' (dominant) since there appear to be independent genetic factors in *B. napus* background (Werner *et al.*, 1995).

MacKay (1977) suggested that introgressed turnip *B. rapa* s-alleles continue to function normally in *B. napus* background. The introduction of new *B. rapa* and *B. oleracea* s-alleles into *B. napus* by introgression appears to be relatively easy as demonstrated by MacKay (1977), Banks and Beversdorf (1994), and Ripley and Beversdorf (1991). At Guelph the focus has been on introgression of non-suppressible s-alleles and we have isolated and introgressed some 30 and 6 s-alleles from *B. rapa* and *B. oleracea*, respectively. Selection of s-alleles was primarily focused on inheritable, strong and stable si expression, as well as on a high response to salt solution or CO_2 to overcome si for s-line maintenance. Isolated *B. rapa* s-alleles were introgressed by backcrossing and plants were scored for si expression at every generation, both by 1) fluorescence microscopy-pollen tube penetration through stylar tissue, and 2) seed set under pollination bags. Crosses were advanced only when the si parent score was excellent or good (Table 1).

TABLE 1. Scoring method for selecting strong and stable s-alleles

Fluorescence Microscopy	Visual examination of seed set on whole bagged plant	si classes*
0 pollen tubes	0 to 10 seeds per plant	excellent
0 pollen tubes	few pods with few seeds each	good
> 15 pollen tubes	10-20+ pods with some seeds	poor
15+ pollen tubes	good seed set	not si
many pollen tubes	full seed set	sc

*flowers of one raceme/plant were treated with salt solution to determine level of selfing and crosses were made with a sc genotype to determine level of fertility.

Homozygosity was achieved by doubled haploid extraction from si plants of advanced backcross lines. At bolting identification of the strongest si individuals was done by pollinating stigmas of newly opened flowers of haploid plants with pollen from diploid plants homozygous for the specific s-allele introgressed, prior to fluorescence microscopy. Selected si doubled haploids were bagged and periodically exposed to CO_2 during flowering to produce seed homozygous for specific s-alleles. By consistent strict selection of individuals with the strongest s-alleles among backcross progeny, the probability exists that systematic elimination of modifier genes that weaken si expression is occurring during backcross cycles. Therefore selection for strong si reaction automatically selects against the incorporation of genes that suppress or modify the si genotype. Complicated hybrid schemes that involve several non-suppressible s-alleles simultaneously reveal the need to fully understand interallelic relationships before incorporation into breeding lines.

GENETIC FACTORS AFFECTING SI EXPRESSION

The effectiveness of a hybrid system depends on a clear understanding of sources of variability. Factors such as s-allele genotype, background genotype, si expression throughout the flowering period and temperature should be evaluated for their specific effects on the outcrossing frequency of both si homozygotes and heterozygotes.

S-alleles introduced into *B. napus* each have a specific pattern of expression and a specific degree of dominance over the native self-compatible phenotype. Introgressed alleles may vary in expression from complete dominance to behaving in a recessive manner in the heterozygous state. Parker (1994) found the major factor contributing to the variation of si expression in crosses, was the s-alleles themselves, which accounted for 82.7% of the variation observed. When utilizing several s-alleles in a hybrid system, dominance relationships among s-alleles must be determined, since a weakening of the si phenotype in the heterozygote would compromise the level of hybridity of the final product.

Cultivar genotype has less influence on si expression (accounting for 7.4% of variation, Parker, 1994) than specific s-alleles, and the observed variation between genotypes in *B. napus* possibly indicates the presence of independent genes modifying or suppressing si expression. Nasrallah and Wallace (1968) and Richards and Thurling (1973) found modifier genes in *B. oleracea* and *B. rapa*. Similarly, Banks and Beversdorf (1994) and Gowers (1989) found significant background influence in *B. napus* with the less dominant s-alleles being affected to a greater degree. For example, Parker (1994) found that cv Westar contributed to a decrease of 7-8% in hybridity, while hybrids with Global increased by 6%. Topas and Regent were relatively neutral in this respect and therefore offer excellent background for new allele evaluation.

Consistent patterns of expression of various s-alleles have been observed during the flowering period in *Brassica* both as homozygotes and heterozygotes. Whether the highest level of hybridity is expressed during the middle of the flowering period (Richards and Thurling, 1973) or towards the end (Parker, 1974), it appears that patterns of selfing/outcrossing were consistent for specific related genotypes suggesting that these patterns are genetically determined, and therefore selection for inbreds with low incidence of selfing should be possible.

Environmental factors are known to have a modifying effect on the s-allele expression in *Brassica*. Higher temperature can cause breakdown in the incompatibility reaction in some genotypes resulting in a higher level of self pollination (Richards and Thurling, 1973).

HYBRID SEED PRODUCTION UTILIZING SUPPRESSIBLE S-ALLELES

Utilization of recessive s-alleles in hybrid production schemes in oilseed *Brassica* has been suggested as a method for maximum cross pollination efficiency in hybrid production fields, coupled with high maintainability of s-lines (Thompson, 1978; Thompson *et al.*, 1983; Werner *et al.*, 1995). This scheme utilizes two lines homozygous for different suppressible s-alleles (e.g. S1 and S2) and a sc pollinator during foundation seed production, producing an sc hybrid product. This three-way cross can be altered to utilize isolines in the first cross to maximize the effect of heterosis (Fig. 1, modified after Thompson *et al.*, 1983; Werner *et al.*, 1995).

Advantages of this system are: 1) suppressible allele breeder seed lines are

maintainable with relative ease since si expression of s-alleles intermediate or low on the dominance scale breaks down readily with salt or CO_2 application, 2) availability of an unlimited number of sc pollinators for foundation seed production, and 3)

Fig. 1.

Breeder seed	Foundation seed	Certified seed
A (S1S1) x ---------- blended seed --------> A^1 (S2S2)	A (S1S2) x ------- strip planted -------> B (SCSC)	AB (S1SC) (S2SC)

certified seed produces sc hybrid plants ensuring maximum seed yield. The disadvantages of this hybrid scheme are twofold. First, rapid inbred development, either by introgression or isolation from existing *B. napus* populations is hindered by the need for progeny testing after each backcross to identify s-allele homozygotes. Recent interest in developing molecular probes for si should facilitate introgression of recessive alleles (Trick and Heinzmann, 1992; Esch and Wrick, 1995). Secondly, the need for strip planting of the sc pollinator rows alternating with si lines is cost inefficient and depends heavily on bee activity.

HYBRID SEED PRODUCTION UTILIZING NON-SUPPRESSIBLE S-ALLELES

Rather cumbersome hybrid production methods have been proposed in vegetable Brassicas such as the 4-way cross using isogenic lines (with 4 alleles) or the triple cross (with 6 alleles). These measures were necessary to facilitate seed multiplication, since the inbred seed was produced by bud pollination. Si inbred seed in oilseed rape is readily increased in isolated field scale multiplications with CO_2 tunnels or salt spray applied by farm machinery, and therefore extra crosses merely to increase seed should not be necessary. Gowers (1975) suggested utilizing introduced s-alleles as a pollination control system in *B. napus* with a combination of si and sc lines. These mixed si/sc hybrid production schemes require strong s-alleles (e.g. S1, S2) that are dominant to the resident sc phenotype in *B. napus* (Fig. 2, modified from Gowers, 1975). A modified 3-way cross varies from this scheme at foundation seed production in that the B line is fully self compatible and strip planting is then required.

Fig. 2. Modified double cross:

Breeder seed	Foundation seed	Certified seed
A (S1S1) x -------- strip planted ----------> A^1 (SCSC)	A (S1SC) x ------ blended seed --------> B (S2SC)	AB (S1S2) (S1SC) (S2SC) (SCSC)
B (S2S2) x -------- strip planted ----------> B^1 (SCSC)		

The advantage of using dominant alleles is that in the 3-way cross only one s-allele is required, while in the double cross seed can be mixed during foundation seed production for higher pollination and land use efficiency. Furthermore, the choice of evaluated non-suppressible s-alleles now is relatively large, providing excellent opportunities for ideal allele combinations. Disadvantages are that during commercial seed production some portion of the plants require obligate outcrossing in order to set seed and in both models the necessity of strip planting during one or both seed increases creates inefficiencies. A true double cross hybrid scheme field tested in *B. rapa* has recently shown that with two pairs each of closely related inbreds and carrying 4 different but complementary s-alleles, a hybrid is produced that sets full seed and requires no strip planting (Kott, unpublished).

At Guelph we are exploring the opportunity to utilize s-alleles at both s-loci simultaneously in order to capture stable and predictable levels of hybridity over a wide range of genotypes and environments. Preliminary studies indicate that interlocus si expression of double heterozygotes is intermediate between corresponding single heterozygotes, that some *B. oleracea* s-alleles exhibit a positive interaction with some *B. rapa* s-alleles, and that some allele combinations show mutual weakening (e.g. S1, S2 from *B. rapa* and Sa, Sb from *B. oleracea*) would eliminate strip planting, provide a greater array of s-alleles to choose from, and perhaps increase level of selfing through mutual weakening of si expression in commercial seed production fields (Fig. 3).

Fig. 3. 4-way cross using 2 s-loci:

Breeder seed	**Foundation seed**	**Certified seed**
A (S1S1)		
x --------- blended seed ----------→	AB (S1S2)	
B (S2S2)		
	x ------- blended seed ---------→	ABCD (S1Sa)
C (SaSa)		(S1Sb)
x --------- blended seed ----------→	CD (SaSb)	(S2Sa)
D (SbSb)		(S2Sb)

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